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Urban salt contamination impact on tree health and the prevalence of fungi agent in cities of the central Lithuania

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ABSTRACT

An analysis of tree health in urban greeneries exposed to winter road salt contamination was carried out in the cities of Alytus and Kaunas, Lithuania, during spring and summer 2009–2014. Trees were assessed for crown dieback, crown defoliation and foliage discolouration. In addition, the prevalence of saprotrophic pathogenic fungi that cause sooty mold disease was assessed in street and recreational plantings. *Tilia cordata* Mill. (small-leaved lime) was found to be the most common tree species among urban deciduous trees. Summarising the tree foliage results, saprotrophic fungi were detected on 16 species plants belonging to 13 genera. Three species of fungal pathogens belonging to two genera, two families, two classes, and two divisions, and 12 species of anamorphic fungi from nine genera were isolated and identified from *Tilia cordata* leaves. The most frequent sooty mold disease agents were *Aspergillus brasiliensis* and *Cladosporium herbarum*. Nonetheless, a weak correlation between salt contamination and lime tree damage by sooty mold was found.

1. Introduction

The leaf surfaces of some plant species are suitable for colonisation and development of saprotrophic fungi, a large number of which belong to the sooty molds. These microscopic fungi form mycelia composed of a black plaque and can also infect buds, fruit and branches. Sooty molds require a sugary substrate with high osmotic pressure for their development. Moreover, they can grow on honeydew secreted by insects of the order *Hemiptera*, suborder *Homoptera*, which includes aphids, whiteflies, soft scales, mealy bugs, leafhoppers and psyllids (Chomnunti et al., 2014). Molds also feed on organic substances secreted by plants (Butin, 2011). The hyphae of separate molds can intertwine on the plant to form heterogeneous colonies, and these may be composed of fungi of the same or different species and sexual and asexual stages (Hughes and Seifert, 2012). Sooty molds can be found on plants from the boreal climate zone to the tropics (Voglmayr et al., 2011); however, their numbers and biodiversity are greatest in the tropics. The mold spores are usually distributed by wind and rain, and sometimes by insects. Spore growth depends on the environmental conditions, particularly on favourable temperature and moisture (Chomnunti et al., 2014).

Some 40 genera (Sinclair and Lyon, 2005) and some 200 species (Chomnunti et al., 2014) of sooty molds have been described, although these numbers vary depending on the fungal systematics used. The most frequently referenced fungus genera are *Capnodium* Mont. (=*Fumago*) (Rupais and Kalnina, 1979), *Torula* Pers. (=*Hormiscium*), *Dematium* Pers. (=*Aureobasidium*) (Chomnunti et al., 2014), *Cladosporium* (Chomnunti et al., 2014), *Triposporium* Corda, *Sarcinomyces* Lindner (Butin, 2011), *Chaetothyrium* Speg., *Euantennaria* Speg., *Metacapnodium* Speg., *Scorias* Fr. (Sinclair and Lyon, 2005). The most widespread temperate zone species described by various authors are *Aureobasidium pullulans* (de Bary, Löwenthal) G. Arnaud and *Cladosporium herbarum* (Pers.) Link (Friend, 1965; Phillips and Burdekin, 1982; Sinclair and Lyon, 2005; Butin, 2011). Vilkaitis (1926) was the first to mention 'leave sooty': 'This is sooty mold, disease agents of which are *Fumago* (*F. vagans* Pers., *F. tiliae* Fuckel), *Apiosporium* spp., *Alternaria* sp., *Cladosporium herbarum* (Pers.) Link, and others. These fungi are saprotrophs developing in secretions of aphids which used to damage the limes.

The prevalence of sooty mold disease agents and the damage they inflict on trees in urban greeneries depend on mold growth conditions and general plant health. In northern regions, road salt (mainly KCl) applied to city streets in winter as a de-icing agent (Jackson and Jobbagy, 2005; Kelly et al., 2008) is one of the most important sources of soil and surface-water contamination (Williams et al., 2000). Chloride contamination of water by road salt

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Table 1
Quantity of study sites and systematic diversity of investigated tree.

City Taxon	Park + forest park		Square	Street
Alytus	7		6	19
Kaunas	9 + 7		8	31
Plant	5 280			2 718
Species	98			33
Genus		57		
Cultivar	18			3

can increase to 36–102 mg L^{-1}, and thus can induce serious damage to urban plantings as well as disturb the functioning of ecosystems in urban settings (Jin et al., 2011). Furthermore, the salt can accumulate in the soil, and hence past use may continue to have adverse effects on existing vegetation (Munck et al., 2010). In addition, salt ions from aerial deposition or salt spray can enter leaves directly via the cuticle, thus damaging vegetative parts. Moreover, salt ions cause osmotic and ionic stress to plants, resulting in dehydration of cells, inhibition of enzymes involved in carbohydrate metabolism, or impairment of photosynthesis (Munns and Tester, 2008). The effects of de-icing compounds could potentially compromise tree disease-resistance.

Effects on the state of tree health as well as the prevalence of sooty mold pathogens in the presence of increasing urban chloride contamination from street salt have not been sufficiently analysed in Lithuania. Therefore, the objectives of this study were: 1) to assess the prevalence of sooty mold, other diseases and pests in street and recreational greeneries exposed to KCl salt contamination in Kaunas and Alytus; 2) to investigate damage to trees caused by sooty molds; 3) to analyse the chemical composition of the foliage under salt contamination.

2. Material and methods

2.1. Study sites and tree specimens

Tree health was monitored in Alytus (54°23′57.9″N, 24°2′48.78″E) and Kaunas (54°53′55.06″, 23°54′25.97″) during the summers of 2009–2014. The aforementioned regions are situated in the southern (4A hardiness zone) and central parts (5 hardiness zone) of Lithuania; the climate is temperate with a mean annual precipitation of 627 mm and a mean summer (winter) temperature of 17 °C (−4 °C). Summer temperatures above 30 °C can occur. Winters can be very cold, with temperatures ranging between freezing and −25 °C in January and February.

In total, 2718 woody plants (belonging to 28 genera, 33 species, 3 varieties) were examined in street plantations, and 5280 woody plants (belonging to 57 genera, 98 species, 18 varieties) in recreational greenery (parks and squares) (Table 1). Tree health was estimated in 7 parks, 6 squares and 19 streets in Alytus; and in 9 parks, 7 forest parks, 8 squares and 31 streets in Kaunas.

The small-leaved lime tree (*Tilia cordata* Mill.) was the most prevalent tree species in both cities, and thus was used for assessment of damage caused by sooty mold and other fungal diseases, salt contamination, crown defoliation, and foliage discolouration.

2.2. Prevalence of sooty mold and other disease agents

The samples for assessment of damage by fungal diseases were collected from stems, leaves, and branches of trees. Fungi agents were identified by disease symptoms and morphological characteristics. The pathogens were isolated from the leaves or bark using the moist chamber method, and they were identified based on their morphological features (Sinclair and Lyon, 2005).

Moist chamber method (Booth 1971): collected branches and leaves injured by fungi were placed in sterilised polythene bags and kept for one day in a refrigerator at 4 °C. The branch or leaf pieces were then placed in Petri dishes on a sterile filter paper moistened with distilled water. The samples were kept for 1–3 days at 24 °C until spores were released from the fruiting bodies. The isolates were cultured by transferring spores onto Petri dishes supplemented with nutrients.

Isolation of pure fungal cultures (Booth, 1971): the samples of branches and leaves were washed with water and again with distilled water. The samples were sterilised in a solution of 3% sodium hypochlorite (NaClO) for 1 min, washed twice for 1 min with sterile distilled water, drained on sterile filter paper, and left to dry for 4 min. The branches were cut into 2–3 × 10 mm pieces, whereas leaves were cut into 25 mm × 25 mm pieces. The pieces were placed in Petri dishes on a nutritional medium containing 2% malt extract agar (MEA). Chloramphenicol (250 mg L^{-1}) for impeding the growth of bacteria was added to the nutritional medium. The Petri dishes were kept in an incubator for 6 days at 28 ± 2 °C. Colonies with different morphological and cultural features were transferred into separate Petri dishes on MEA nutritional medium.

Fungi were identified according to the symptoms of diseases and fungal morphological features defined by descriptors (Carmichael et al., 1980; Domsch et al., 1980; Ellis, 1976). Fungus ontology was described according to Index fungorum (2014). Other tree pests were identified according to Hartmann et al. (2005).

The intensity of disease was evaluated visually for each tree using a five-point scale: 1– up to 10% of the surface area of sampled leaves, branches and trunks injured; 2– from 11% to 30%; 3– from 31% to 60%; 4– from 61% to 80%; and 5– from 81% to 100%.

The mean grade of damage was calculated using the following formula (Šurkus and Gaurilčikienė, 2002):

$$V = \frac{\sum_{i=1}^{5} (n_i \cdot b_i)}{N}, \quad (1)$$

where: V – the average grade of damage; n_i – number of plants damaged to the same grade and the sum of products of the i_{th} grade; b_i – the numeric value of the i_{th} grade; N – number of checked plants.

2.3. Chloride inputs

The sources of chloride input are winter road and street technical salts: sodium chloride (NaCl) and potassium chloride (KCl). The lime tree (*Tilia cordata*) was determined to be the most prevalent tree in Alytus city; therefore, it was selected for assessment of salt contamination and other stress factor-induced damage along four main streets (A. Juozapavičiaus, Naujoji, Pulko and Kauno) that had high traffic levels in Alytus. Trees in Jaunimo Park served as a control (without salt application).

Additionally, multiple foliage samples from lime trees prevalent in selected streets were taken for assessment of chemical content at the Agrochemical Laboratory of Lithuanian.

2.4. Tree damage surveys

Tree morphological-physiological surveys were conducted beginning in late spring and continued throughout the summer months during 2009–2014. The international forest monitoring methodology (UN/ECE, 1998), adapted for the monitoring of urban greenery, was used (Table 2).

Trees that were used for the assessment of crown dieback, crown defoliation and foliage discolouration were selected along four main streets in Alytus (A. Juozapavičiaus, Naujoji, Pulko and Kauno) that had high traffic volumes.